Genetics of floral traits of *Jaltomata procumbens* (Solanaceae)

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Abstract. Understanding the genetic control of key reproductive traits/characters facilitates better understanding of systematics and evolution. Accordingly, we employed hybridization studies to investigate the Mendelian genetics of eight floral traits of two divergent populations of *Jaltomata procumbens*, which appear to have undergone an evolutionary shift in mating system. Marked phenotypic differences allowed us to analyze the genetics of morphological differentiation. Flowers per inflorescence, sepal, petal, filament, and anther length, and stigma diameter all show continuous variation, and therefore may be polygenic. The bimodal F_2 distributions of the extent of nectar guides (petal spots) and staminal pubescence suggest that each is controlled primarily by a major gene. Our data supports the widespread idea that most phenotypic variation is controlled by numerous genes each having small effect, but also that a difference between two divergent populations is sometimes primarily due to one gene having a large effect.

Keywords: Nectar guides, petal spots, polygenic traits, functional traits, transgressive segregation.

Gottlieb (1984) suggested that the genetic basis of morphological differences between species can be regarded as essentially the same as those within species. Thus, studying genetic divergence within species may contribute to improved understanding of speciation, and intraspecific variation is a neglected aspect of speciation genetics (Lexer & Widmer, 2008). Approaches to the study of speciation can be retrospective (after the fact) or during divergence (Via, 2009). A major advantage of studying speciation in process is that one's conclusions are not confounded by post-speciation evolution. A criticism of this approach is that we don't know whether the variation we see within species will ever lead to speciation (Via, 2009). However, if there is a continuum of divergence, we may be able to study speciation at an early stage. Many populations differ by features associated with their flowers (Levin, 2000). Clarifying the genetic control of these reproductive traits/characters will facilitate a better understanding of evolution (Barrett, 2010), and may provide insights into the speciation process and/or data relevant to species delimitation.

Here, we use hybridization experiments to investigate the Mendelian genetics of variation in floral morphology of functional traits (e.g., Díaz et al., 2016) in two morphologically divergent populations of *Jaltomata procumbens* (Cav.) J. L. Gentry (Solanaceae). The subject of previous research by the first author (Mione, 1992; Mione & Yacher, 2005), *J. procumbens* is a common, phenotypically variable, diploid, herbaceous, perennial distributed from Arizona, USA, to Ecuador. It is commonly found in disturbed habitats, including as a weed in agricultural fields where it is usually tolerated because of its edible fruits (Davis & Bye, 1982). The flowers have a green corolla with darker green petal spots, yellow anthers, and nectar droplets that accumulate on the base of the corolla. The flowers are visited by bees in Mexico (Williams, 1985) and are self-compatible (Mione & Yacher, 2005).

The two populations we studied show notable differences in floral morphology, to such an extent that some taxonomists might consider them to be insipient species. One population has relatively large flowers exhibiting herkogamy (anthers spatially separated from the stigma [Fig. 1A], but later connivent), while the other has smaller flowers lacking herkogamy (stamens always connivent, anthers at the stigma). In addition to

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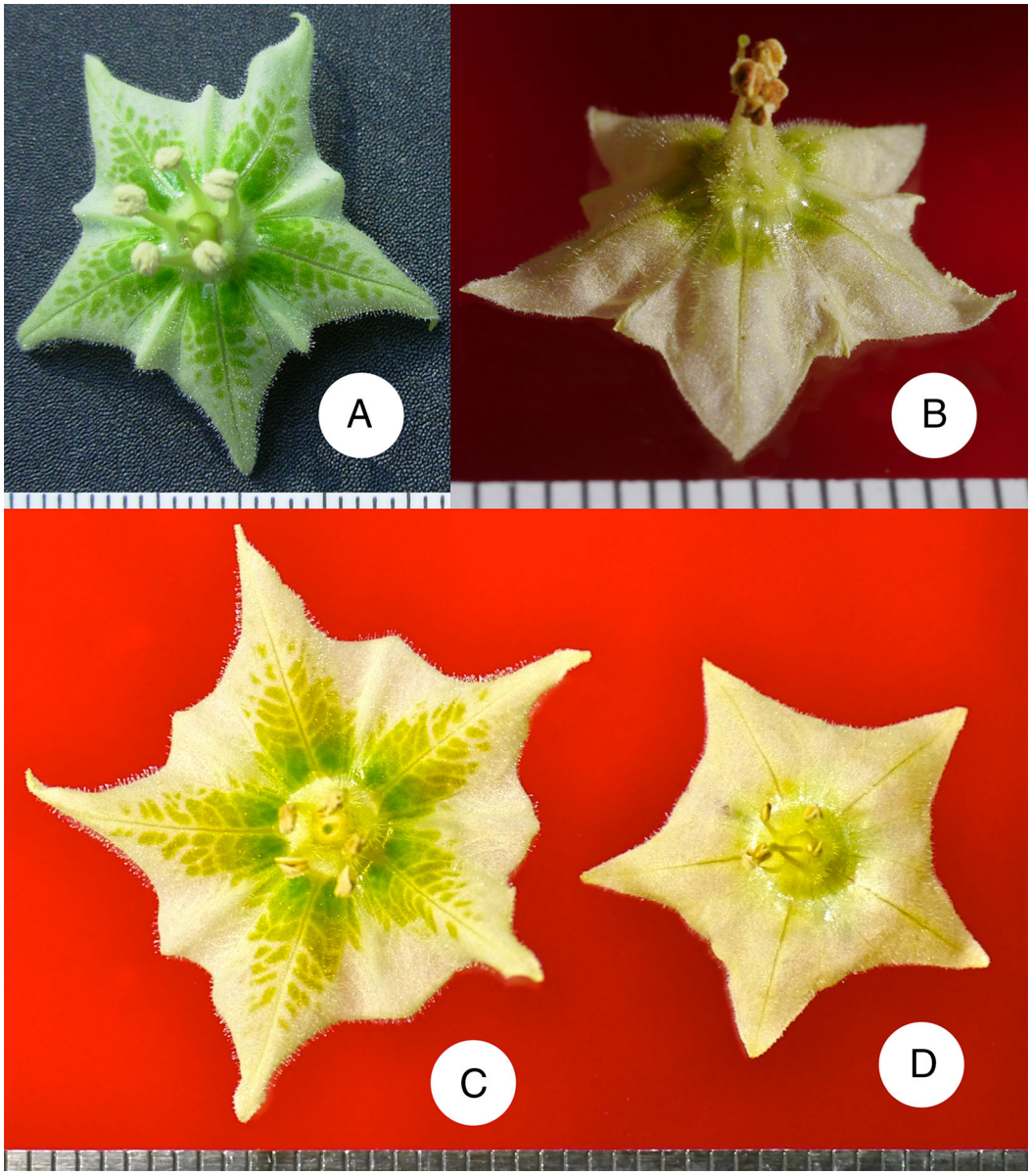

Fig. 1. **A.** *Jaltomata procumbens* P_1 derived from accession *599*. Petal spots extend from the base most of the way to the petal lobe. Units are mm. **B.** P_1 derived from accession *321*. Basal petal spots. Units are mm. **C.** Petal spots of some F_2 plants were made up of two different green hues: basal spots are darker green than spotting that extends most of the way towards the petal's lobe tip. Units are mm. **D.** F_2 transgressive segregant having less petal spotting than the minimum observed in P_1 *321*. Units are mm. Photos by T. Mione.

the eight traits we selected for genetic analysis (Table 1), cultivated plants derived from seeds collected from the two populations differ consistently in the following (with the trait of accession *599* listed prior to that of *321)*: leaf pubescence (glabrous versus hairy), leaf margin (entire to repand versus toothed to repand), disparity in stamen elongation rate within flowers (equal rates versus unequal rates), pollen size (30–35 μm versus 25.5–28.8 μm), ovules per ovary (114–193 versus 66–108), maximum fruit width (18.2 mm versus 9 mm), patterning of purple pigmentation

TABLE 1. Floral traits measured.

No.	Traits	Definition	Units	Developmental stage
1	**Flower number**	Number of flowers per inflorescence including flowers, flower buds, and pedicel scars		Inflorescences with at least one open flower
2	**Sepal length**	Distance of flattened structure from the base to the tip of the lobe	mm	Open flowers
3	**Petal length**	Distance of flattened structure from the base to the tip of the lobe	mm	Hermaphroditic stage (after anther dehiscence)
4	**Extent of petal spots**	Length of the petal zone having spots divided by petal length x 100	%	Hermaphroditic stage (after anther dehiscence)
5	**Filament length**	Distance from base to apex of staminal filament	mm	Hermaphroditic stage (after anther dehiscence)
6	**Extent of filament pubescence**	Length of portion of filament having hairs divided by filament length x 100	%	Hermaphroditic stage (after anther dehiscence)
7	**Anther length**	Distance from base to apex	mm	Open flowers, prior to anther dehiscence
8	**Stigma diameter**	Longest dimension of the stigmatic surface	mm	Open flowers

on calyx subtending mature fruits (solid versus radial markings), seed size (1.5–1.7 x 1.2–1.4 versus 1.3–1.5 x 1.0–1.2), number of seeds per fruit (32–180 versus 24–63), and degree and timing of corolla closure (full closure in late afternoon versus no closure or partial closure hours later) (T. Mione, unpubl. data).

We have grown for study 22 different collections of *Jaltomata procumbens* from three countries. All but one (see below) exhibits herkogamy, and thus we assume mixed mating predominates in *J. procumbens*, and that the morphology of the larger-flowered population studied here promotes outcrossing, while that of the small-flowered population (the exception) is associated with increased and/or obligate selfing (Moore & Lewis, 1965). Thus, our study was undertaken in the context of a hypothetical shift in mating system, which we assume has contributed to the observed morphological divergence between the two focal populations.

Materials and methods

Plant materials.—Approximately 50 to 75 seeds from one plant of each accession were collected from two natural (non-cultivated) Mesoamerican populations of *J. procumbens* at similar altitudes but located some 1500 km apart. Accession *599* was collected in 1994 in Morelos, Mexico at 2230 m elevation from a population displaying wild type morphology (i.e., relatively large flowers and herkogamy) and is vouchered by the herbarium specimen *T. Mione & R. Bye 599* (CONN). Accession *321* was collected in 1995 in Chiquimula, Guatemala at 1930 m from a population displaying floral morphology atypical for the species (i.e., relatively small flowers lacking herkogamy) and is vouchered by *D. Spooner et al. 7038* (AGUAT, BIGUA).

The two seed accessions, stored at 10 C, were germinated at room temperature. The plants derived from both accessions abundantly self-set fruits in the greenhouse in the absence of pollinators. Three true-breeding generations of each accession were grown in pollinator-free greenhouses, and then two individuals derived from each of the two accessions were selected to be used as the parental generation (P_1) for hybridization.

Crosses.—Hybridizations of the P_1 plants were done manually, with P_1 *321* randomly chosen to supply pollen, and protogynous flowers (i.e., fully open but prior to anther dehiscence) of P_1 *599* randomly selected to receive pollen. Flowers were emasculated at the time of pollination to avoid subsequent autogamy. Following five manual hybridizations between the P_1 plants, all of which resulted in fruit-set, seeds from one randomly chosen fruit were planted to produce the F_1 generation. Only six seeds germinated of several dozen planted, and one died, giving an F_1 of five plants. Dozens of self-set fruits were formed on

the F_1 plants and from these, seeds were randomly selected to form the F_2 . Sample sizes in the F_2 were unequal, however, because some seedlings were eaten by herbivores, resulting in many more F_2 plants from seeds set on some F_1 plants and few from others. The number of F_2 plants from a given F_1 plant ranged from 2 to 50, for a total of 161 F_2 plants.

Measurements.—Measurements of eight floral traits were made on the P_1, F_1, and F_2 generations in consecutive years (2012–2014) on plants grown in a University of Connecticut greenhouse, an environmentally controlled, pollinator-free environment. The traits chosen are important in pollination and mating systems and are used by some authors as characters for distinguishing species (Ornduff, 1969; Anderson et al., 2002). The traits and their definitions are listed in Table 1. Stamens and stigmata where measured with the aid of a microscope from excised structures. Each trait was measured once on a given flower, two to six times per plant (Table 2). Measurements were nearly evenly distributed among the plants of each generation. Sample sizes for each of the measurements are given in Table 2.

Data Analysis.— Means, ranges and standard deviations were calculated for all measurements of the eight floral traits for P_1 plants, as well as for the F_1 and F_2 generations (Table 2). For the F_2 generation only, the coefficient of variation and skewness were calculated and normality was tested. Frequency distributions were generated for each of the traits in the F_2 based on all F_2 measurements, and also with a subset of the F_2 data using only those F_2 plants derived from seeds set on an individual F_1 plant (frequency distributions not shown). To gain insight into the genetic integration of traits, phenotypic correlations among the F_2 means for the eight focal traits were examined using Pearson and Spearman correlations. The software Prism 6.0f (GraphPad Software, Inc., www.graphpad.com) was used to perform normality tests (D'Agostino and Pearson omnibus), to compute correlations, and to make frequency distributions. The number of loci influencing trait variation was estimated using the Castle Wright estimator (Lynch & Walsh, 1998, p. 233; Hartl & Clark, 2007, equation 8.50, p. 453), which assumes that all genes have equal effects, no dominance, and are unlinked. The number of loci for sepal length was not estimated because, surprisingly, the F_1 phenotypic variance was higher than the F_2 variance.

Results

Parental and F_1 generations.—Statistically significant differences between the P_1 plants derived from the two accessions were observed in the eight measured traits (for all eight T-tests, $P = 0.0003$ or lower). Relative to P_1 *321*, P_1 *599* had longer sepals, petals, filaments and anthers, more extensive petal spots, and larger stigmata, but a lower flower number, and less extensive filament pubescence. The mean of the observations for the F_1 generation were intermediate between the means of the parents for all traits. However, for several traits the F_1 resembled to a greater extent one or the other parent. For example, the F_1 mean of trait 4 (extent of petal spots) was much closer to the mean of P_1 *599*, whereas for trait 6 (extent of filament pubescence) the F_1 mean more closely approached that of P_1 *321*.

F_2 generation.— The F_2 was derived completely from selfing: dozens of self-set fruits (flowers are autogamous if not emasculated) developed on the five F_1 plants. The shapes of the F_2 distributions were similar regardless of whether they were based on subsets of the F_2 data for plants derived from individual F_1 plants (data not shown) or on the pooled data. Thus, we report results for the pooled data only (Fig. 2). Traits 1 (flower number), 3 (petal length), 5 (filament length), and 7 (anther length) were normally distributed in the F_2, whereas traits 2 (sepal length), 4 (extent of petal spots), 6 (extent of filament pubescence), and 8 (stigma diameter) failed the normality test (Table 2). Stigma diameter in the F_2 was skewed toward the parent (*321*) having smaller stigmata.

Traits 4 and 6 were skewed in the F_2 toward the parents having more extensive petal spots (*599*) and more extensive filament pubescence (*321*), respectively. Bimodal distributions were evident in the F_2 for both of these traits (Fig. 2). Petal spots (trait 4) were either distributed from the petal base out to 10–25% toward the lobe tip or from the petal base out to 35–76% toward the lobe tip (Fig. 2). Slightly over 25% (43/161) of the F_2 plants had only basal petal spots, not significantly different from a ratio of 1:3 (two-tailed X^2=0.251, df=1, P=0.617). In the case of extent of filament pubescence (trait 6), 38–41/161 (~25%) of the F_2 plants fell into the left distribution, and 123–120/161 (~75%) into the right distribution. The result for trait 6 was again not significantly

Table 2. Floral traits of the parental, F_1, and F_2 generations.

Trait no.*	**Parent *599*** • mean • range • s.d. • no. plants/ no. obs.	**Parent *321*** • mean • range • s.d. • no. plants/ no. obs.	**F_1 gen.** • mean • range • s.d. • no. plants/ no. obs.	**F_2 gen.** • mean • range • s.d. • no. plants/ no. obs.	**F_2 gen.** • coeff. of variation • passed normality? • skewness
1	5.8 5–6 0.447 2/5	11.3 10–12 0.816 2/6	7.9 6–10 0.852 5/20	7.6 4–11.3 1.297 161/359	17.1% Yes, P=0.7608 -0.1091
2	6.6 6–8 0.605 2/10	2.8 2–3.5 0.382 2/13	4.1 3.3–4.9 0.558 5/19	4.2 3–6 0.500 161/435	11.9% No, P=0.0078 0.392
3	14.6 12.5–16 1.497 2/6	9.1 8–10 0.582 2/8	11.6 10.5–13.4 0.991 5/18	10.9 8.5–14 1.145 160/328	10.5% Yes, P=0.4515 0.2128
4	57% 40–69% 7.939 2/9	24% 17–30% 5.12 2/8	45% 39–54% 3.884 5/18	41% 10–76% 16.89 161/328	41% No, P<0.0001 -0.3013
5	6.6 5.5–8 0.845 6/10	5.5 4.5–7 0.567 7/18	6.0 5–6.6 0.501 5/19	5.6 4.25–7.05 0.537 161/330	9.6% Yes, P=0.3708 0.2643
6	23% 21–27% 2.271 6/10	71% 64–80% 4.162 7/18	61% 45–80% 8.280 5/19	51% 18–85% 16.79 161/330	33% No, P=0.0001 -0.5789
7	2.5 2.1–2.7 0.26 2/8	1.6 1.4–1.7 0.12 2/6	2.0 1.7–2.35 0.15 5/23	2.05 1.65–2.58 0.175 157/352	8.5% Yes, P=0.6109 0.1734
8	0.59 0.45–0.74 0.1 2/9	0.27 0.21–0.33 0.04 4/14	0.47 0.37–0.58 0.06 5/23	0.395 0.256–0.636 0.645 161/366	16.4% No, P=0.0003 0.7331

*Trait no. corresponds to the numbers given in Table 1.

different from a 1:3 ratio (two-tailed $X^2 = 0.019$, df = 1, P = 0.891 if the fifth bin, i.e. "40", is treated as a part of the left distribution; two-tailed $X^2 = 0.168$, df=1, P=0.682 if the 40% bin is treated as part of the right distribution). However, the F_2 data for both traits is notably variable, with some individuals being transgressive with respect to the P_1 (Fig. 2). For example, 21/161 (13%) of the F_2 plants had petal spotting less extensive than the minimum observed in the *321* parents (Fig. 1D), and five F_2 plants (3%) had petal spotting more extensive than the maximum observed in the *599* parents. Moreover, the presence of petal spots of two different green hues in some individuals of the F_2 was not observed in any of the P_1 plants (Fig. 1c).

Traits 1, 3, 5 and 8 were estimated to be influenced by 0.7, 2.1, 3.7, 1.8 and 3.4 loci respectively. We did not estimate the number of loci influencing the extent of petal spots (trait 4) or the extent of filament pubescence (trait 6) because the F_2 frequency distributions were bimodal, thus violating an important assumption of the Castle Wright estimator.

More than half (15/28) of the pair-wise F_2 trait correlations are significant (Table 3). Size traits are significantly positively correlated, as is extent of petal spots with both anther and filament length. Flower number is negatively correlated

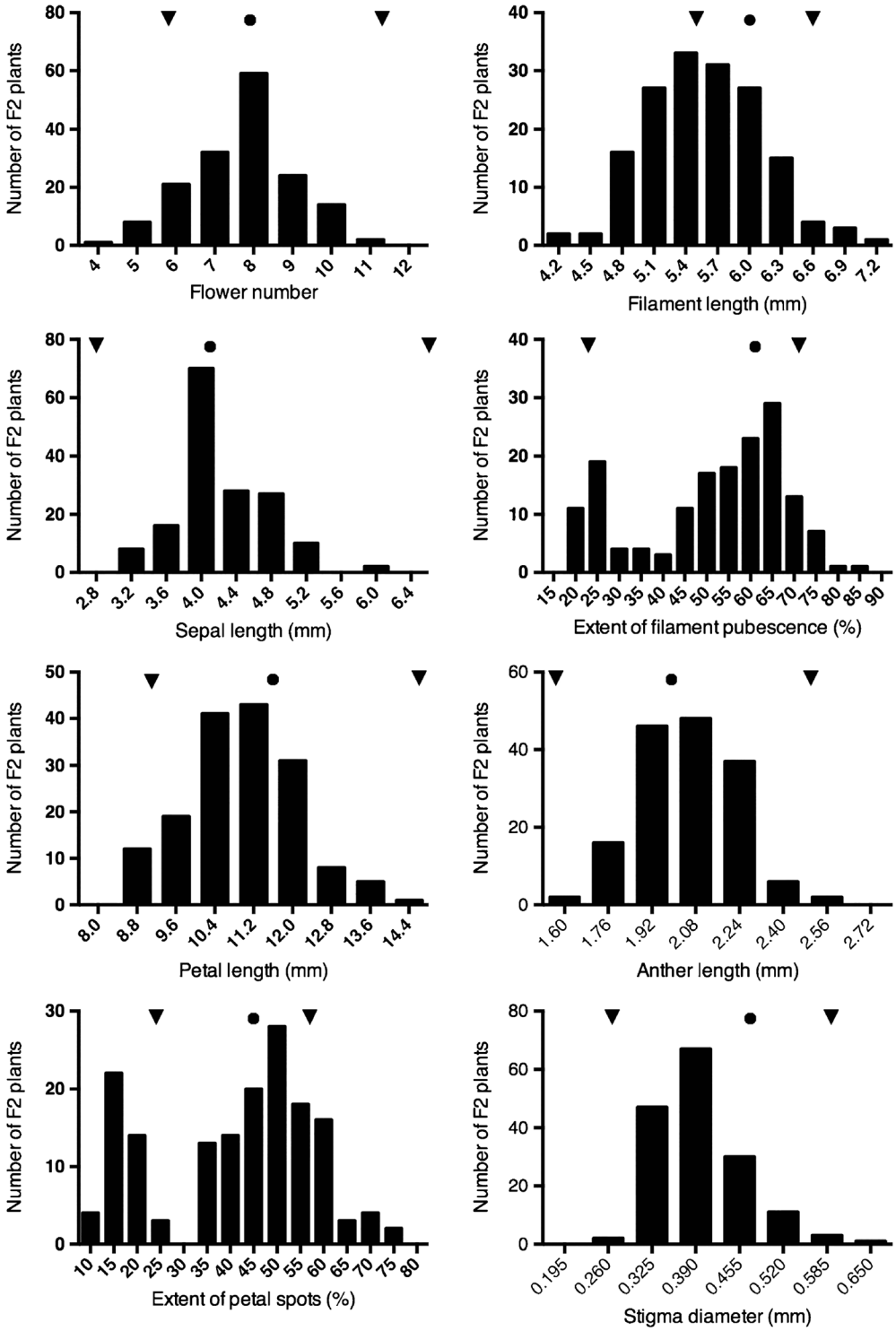

FIG. 2. F_2 frequency distributions. Triangles and circles mark the mean values for the parental (P_1) and F_1 generations, respectively. The triangle for P_1 *321* is nearest the Y axis for all traits plotted except flower number and extent of filament pubescence.

Table 3. Phenotypic correlation among traits measured on plants of the F_2 generation[a].

	2	3	4	5	6	7	8
1	0.056	- 0.047 -	-0.030 -	- 0.054 -	- 0.042 -	- 0.017 -	- 0.062 -
2		*** 0.598 ***	-0.113 -	*** 0.278 ***	- 0.012 -	*** 0.474 ***	*** 0.303 ***
3			0.102	*** 0.402 ***	-0.059	*** 0.524 ***	*** 0.350 ***
4				*** 0.328 ***	* 0.196 **	** 0.253 **	- 0.072 -
5					** 0.254 ***	*** 0.397 ***	** 0.207 *
6						- 0.005 -	** -0.249 *
7							*** 0.378 ***

[a] The column and row headings refer to the trait numbers given in Table 1. P values are summarized as * P = .01 to .05; ** P = .001 to .01; *** P < 0.001, with Pearson levels of significance summarized above the Pearson coefficients and Spearman levels of significance below the Pearson coefficients.

with all of the size traits but the correlations are not statistically significant.

Discussion

Our data suggest clear differences in the genetic control of floral traits in *Jaltomata procumbens*, a result in general agreement with previous studies of other angiosperms, which have documented extensive variation in the genetics of floral traits (Grant, 1975). In the following discussion, traits are grouped together that appear to display similar patterns of genetic control.

Traits 1 (flower number), 3 (petal length), 5 (filament length), 7 (anther length).—These traits were intermediate between the parents in the F_1, with the F_1 mean essentially equidistant between the parent means, and displayed unimodal, normal distributions in the F_2. We interpret this pattern as suggestive of polygenic inheritance (without allelic dominance), although flower number was estimated to be under single locus control by the Castle Wright estimator.

Our results for petal length are similar to those obtained for *Clarkia* (Moore & Lewis, 1965; Gottlieb & Ford, 1988) and *Capsicum* (Lippert et al., 1966). Moore and Lewis (1965) hybridized self-pollinating and outcrossing races of *Clarkia xantiana*, and described the inheritance of flower size as multifactorial.

Traits 2 (sepal length) and 8 (stigma diameter).—These traits had F_1 means that were intermediate but displaced from the median relative to the P_1 means, and displayed non-normal, relatively skewed, unimodal distributions in the F_2. We interpret this pattern as either developmental noise or indicative of polygenic inheritance with one or more dominant alleles. Our results for stigma diameter are similar to those summarized by Grant (1975) who studied stigma length by hybridizing races of *Gilia capitata*. In agreement with our data, stigma length in *G. capitata* was skewed toward the parent having smaller (shorter) stigmata in the F_2, with the degree of skew in both his data and ours suggesting that dominant alleles exist in only about one out of three of the additive genes involved (Grant 1975, pp. 181 and 189).

Traits 4 (extent of petal spots) and 6 (extent of filament pubescence).—These traits both displayed means in the F_1 that with respect to the P_1 means, much more closely resembled the parent with more extensive spots (*599*) and

more extensive filament pubescence (*321*). In the F_2 the distributions are bimodal, with the individuals proportioned among the two parts of the distribution in an approximately 1:3 ratio, but also with some individuals displaying transgenic variation with respect to the parents. These results are compatible with control by a single major gene with one allele dominant over the other (Grant, 1975). We presume the major locus to be homozygous in the parents and heterozygous in the F_1. With independent assortment within a major gene having a dominant allele, the prediction for the F_2 is that 25% of the plants will have petal spots as extensive as those of one parent, an outcome in accordance with our observations. However, broad variation among individuals within the two parts of the distributions of both traits reflects either the influence of genes with minor effect or developmental noise. Substantial environmentally driven phenotypic variation among F_2 plants is unlikely since they were all grown in a common greenhouse environment.

Transgressive segregation results if within each parent, at different loci, there are alleles having opposite effects, and these are recombined into novel combinations in the F_2 (Grant, 1975, p. 184; Lynch & Walsh, 1998, p. 477). The genetic architecture that allows transgressive segregation is common (Rieseberg et al., 2003). The presence of petal spots of two different green hues in some individuals of the F_2 (Fig. 1c), a state not seen in the P_1 (photos were not taken of the F_1 flowers), is suggestive of additive gene action.

The petal spots in *Jaltomata procumbens* are presumably nectar guides, visible to the unaided eye as darker green pigment on a lighter green background. Nectar guides benefit both the plants that have them and their pollinators (Leonard & Papaj, 2011). Despite recent advances in our understanding of nectar guides/petal spots (Martins et al., 2013), few researchers have investigated their genetics. In *Gossypium hirsutum* and *G. barbadense* crosses within species show control by a single gene with the petal spots allele dominant over the spotless allele (Harland, 1936). However, interspecific crosses of spotted x spotless resulted in continuous variation in the F_2, due to different modifier genes (Harland, 1936; Grant, 1981, p. 100). In *Clarkia gracilis*, each of the four subspecies has a unique petal spot pattern, and inheritance of petal spots is governed by two major genes, one a suppressor epistatic to the other (Gottlieb & Ford, 1988; Martins et al., 2013). In *Capsicum*, "yellow petal spot" is dominant in interspecific crosses (Lippert et al., 1966). In *Brassica*, the nectar guide is the UV absorbing center of the flower, and in a cross of parents that differed significantly in the proportion of the floral area occupied by the nectar guide, a unimodal, approximately normal distribution was observed in the F_2 (Kobayashi et al., 2006).

Staminal pubescence may decrease insect access to the ovary (see Hanley et al., 2007), increase the attractiveness of flowers, and/or provide a "foothold for visitors" (Fægri & van der Pijl 1979, p. 84). In at least one variety of cultivated tomato, staminal pubescence was shown to be important in reproduction (Rick, 1947), with self-pollination resulting when hairs bind anthers together. But when anther hairs are absent, a large decrease in fruit set was observed. In that study, the presence of staminal hairs was determined by a dominant allele, with partial suppression being a recessive condition.

Filament pubescence varies greatly among *Jaltomata* species, and consequently authors have used this trait in keys and descriptions of species (Macbride, 1962; Mione et al., 1993, 2001, 2007, 2011, 2013, 2015a, 2015b; Leiva González et al., 2008, 2013, 2016). In *J. procumbens*, staminal hairs are unpigmented, while in several South American *Jaltomata* species they are intensely purple, sometimes contrasting with the color of the filament.

Trait correlations.—The positive correlation of size traits with each other in *Jaltomata procumbens* (Table 3) suggests that a single genetic system may control size in multiple floral organs (Macnair & Cumbes, 1989). The negative correlations of both flower number and the extent of filament pubescence with the size traits was expected given that the parent having smaller floral organs had both significantly higher flower number and significantly more pubescent filaments. Phenotypic correlations in the F_2 generation (Table 3) are almost all in the direction of the parental trait combinations, as reported by Clausen & Hiesey (1958, p. 116), due to genetic linkage and or pleiotropy (Grant, 1975, p. 338). The correlation between the extent of petal spots and anther length may be due to linkage given that these traits seem unlikely to be developmentally related.

In a separate study based on midparent midoffspring regressions (T. Mione, unpubl. data), the heritability of all of these traits was quite high, usually about 1, based on data from plants grown in a greenhouse where environmental variation was highly reduced. When the heritability of a trait is high, a phenotypic correlation is a reasonable substitution for a genetic correlation. Indeed, Waitt and Levin (1998) concluded that phenotypic correlations in plants are a good indicator of "their genetic counterparts."

Conclusion.—The interfertility of the two parent (P_1) populations suggests that wholesale genome reorganization has not taken place during the evolution of 19 trait differences (eight studied here), and that morphological differentiation has not been accompanied by the evolution of cross-incompatibility. Sixteen *Jaltomata* species tested to date are self-compatible (SC)(Mione, unpubl. data), and the cross-compatibility of the parents is concordant with SC species tending to be more cross-compatible than self-incompatible species (Anderson, 1977; Whalen & Anderson, 1981; Baek et al., 2015). The F_1 plants abundantly self-set fruits containing seeds that germinated readily, and F_2 plants set fruits as well, suggesting the absence of outbreeding depression (Waser & Price, 1994) and the possibility of selection having favored the ability to outbreed (Solbrig, 1968, p. 93). This study demonstrates, within one variable species, that if the parent populations were to hybridize, transgressive variation may result, a potential source of evolutionary novelties (Rieseberg & Willis, 2007).

We also demonstrated that for the traits analyzed, the estimated number of loci affecting trait variation ranged from one to four. Gottlieb (1984) discussed two categories of morphological traits in plants: 1) those controlled by one or two genes, tending to be presence/absence, structure, shape and architecture traits, and 2) polygenic traits including length, number and weight features. The size traits we studied all appear polygenic, concordant with Gottlieb's observations. However, the two 'extent' traits, petal spots and filament pubescence, do not fit neatly in either category. The data for these traits appear to support Mather and Jinks' (1982, p. 38) observation that "variation may at the same time be due partly to major gene differences and partly to a polygenic system."

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Literature cited

Anderson, G. J. 1977. The variation and evolution of *Solanum* section *Basarthrum*. II. Brittonia 29: 116-128.

——, S. D. Johnson, P. R. Neal & G. Bernardello. 2002. Reproductive biology and plant systematics: The growth of a symbiotic association. Taxon 51: 637– 653.

Baek, Y. S., P. A. Covey, J. J. Petersen, R. T. Chetelat, B. McClure & P. A. Bedinger. 2015. Testing the SI x SC rule: Pollen-pistil interactions in interspecific crosses between members of the tomato clade (*Solanum* section *Lycopersicon*, Solanaceae). American Journal of Botany 102: 302–311.

Barrett, S. C. H. 2010. Understanding plant reproductive diversity. Philosophical Transactions of the Royal Society B 365: 99–109. doi 10.1098/rstb.2009.0199.

Clausen, J. & W. M. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecological races. Carnegie Institution of Washington Publication 615.

Davis, T. IV & R. A. Bye, Jr. 1982. Ethnobotany and progressive domestication of *Jaltomata* (Solanaceae) in Mexico and Central America. Economic Botany 36: 225–241.

Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. C. Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. J. Wright, S. N. Sheremet'ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha & L. D. Gorné. 2016. The global spectrum of plant form and function. Nature 529: 167–171. doi 10.1038/nature16489.

Fægri, K. & L. van der Pijl. 1979. The principles of pollination ecology, 3rd edition. Pergamon Press, Oxford.

Gottlieb, L. D. 1984. Genetics and morphological evolution in plants. The American Naturalist 123: 681–709.

—— & V. S. Ford. 1988. Genetic studies of the pattern of floral pigmentation in *Clarkia gracilis*. Heredity 60: 237–246. doi 10.1038/hdy.1988.37.

Grant, V. 1975. Genetics of flowering plants. Columbia University Press, New York.

——. 1981. Plant speciation, 2nd edition. Columbia University Press, New York.

Hanley, M. E., B. B. Lamont, M. M. Fairbanks & C. M. Rafferty. 2007. Plant structural traits and their role in anti-herbivore defense. Perspectives in Plant Ecology, Evolution and Systematics 8: 157–178.

Harland, S. C. 1936. The genetical conception of the species. Biological Reviews 11: 83–112.

Hartl, D. L. & A. G. Clark. 2007. Principles of population genetics, 4th ed. Sinauer Associates, Inc., Sunderland, Massachusetts.

Kobayashi, K., Y. Yoshioka, A. Horisaki, S. Niikura & R. Ohsawa. 2006. Estimation of heritability of the nectar guide of flowers of *Brassica rapa* L. Breeding Science 56: 75–79.

Leiva González, S., T. Mione & L. Yacher. 2008. Dos nuevas especies de *Jaltomata* Schlechtendal (Solanaceae) del norte del Perú. Arnaldoa 15: 185–196.

——, —— **&** ——**.** 2013. *Jaltomata huancabambae* y *Jaltomata incahuasina* (Solanaceae) dos nuevas especies del norte del Perú. Arnaldoa 20: 265–280.

——, —— **&** ——**.** 2016. Especies de *Jaltomata* Schltdl. (Solanaceae) con néctar rojo, tres nuevas combinaciones nomenclaturales y un nuevo taxón del noreste de Perú. Arnaldoa 23: 21–98.

Leonard, A. S. & D. R. Papaj. 2011. 'X' marks the spot: The possible benefits of nectar guides to bees and plants. Functional Ecology 25: 1293–1301.

Levin, D. A. 2000. The origin, expansion and demise of plant species. Oxford Series in Ecology and Evolution, Oxford University Press.

Lexer, C. & A. Widmer. 2008. The genic view of plant speciation: Recent progress and emerging questions. Philosophical Transactions of the Royal Society B-Biological Sciences 363: 3023–3036. doi 10.1098/rstb.2008.0078.

Lippert, L. F., P. G. Smith & B. O. Bergh. 1966. Cytogenetics of the vegetable crops. Garden Pepper, *Capsicum* sp. Botanical Review 32: 24–55.

Lynch, M. & B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, Massachusetts.

Macbride, J. F. 1962. Solanaceae. Publications of the Field Museum of Natural History. Botanical series 13, Part V–B, No. 1. [*Jaltomata* as *Saracha* and *Hebecladus*].

Macnair, M. R. & Q. J. Cumbes. 1989. The genetic architecture of interspecific variation in *Mimulus*. Genetics 122: 211–222.

Martins, T. R., J. J. Berg, S. Blinka, M. D. Rausher & D. A. Baum. 2013. Precise spatio-temporal regulation of the anthocyanin biosynthetic pathway leads to petal spot formation in *Clarkia gracilis* (Onagraceae). New Phytologist 197: 958–969. doi 10.1111/nph.12062.

Mather, K. & J. L. Jinks. 1982. Biometrical genetics: The study of continuous variation, 3rd ed. Chapman and Hall, London.

Mione, T. 1992. Systematics and evolution of *Jaltomata* (Solanaceae). Ph.D. thesis. University of Connecticut, Storrs, CT.

——**, G. J. Anderson & M. Nee**. 1993. *Jaltomata* I: Circumscription, description and new combinations for five South American species. Brittonia 45: 138–145.

——**, D. Mugaburu & B. Connolly**. 2001. Rediscovery and floral biology of *Jaltomata biflora* (Solanaceae). Economic Botany 55: 167–168.

——**, S. Leiva González & L. Yacher.** 2007. Five new species of *Jaltomata* (Solanaceae) from Cajamarca, Peru. Novon 17: 49–58.

——, —— **&** ——**.** 2013. *Jaltomata spooneri* (Solanaceae): A new species of southern Peru. Phytologia 95: 167–171.

——, —— **&** ——**.** 2015a. Two new Peruvian species of *Jaltomata* (Solanaceae, Solaneae) with red floral nectar. Brittonia 67: 105–112. doi 10.1007/s12228-014-9360-2.

——, —— **&** ——**.** 2015b. *Jaltomata sanmiguelina* (Solanaceae), a new species from Northern Peru. University Proceedings, Volga Region, Natural Sciences, Article 1315.

——, ——, —— **& A. Cameron**. 2011. *Jaltomata atiquipa* (Solanaceae): A new species of southern Peru. Phytologia 93: 203–207.

—— **& L. Yacher**. 2005. *Jaltomata* (Solanaceae) of Costa Rica, Pp. 117–130. *In:* R. C. Keating, V. C. Hollowell, and T. B. Croat [eds.], A festschrift for William G. D'Arcy, the legacy of a taxonomist. Monographs in Systematic Botany from the Missouri Botanical Garden Press, Volume 104.

Moore, D. M. & H. Lewis. 1965. The evolution of self-pollination in *Clarkia xantiana*. Evolution 19: 104–114. doi 10.2307/2406299.

Ornduff, R. 1969. Reproductive biology in relation to systematics. Taxon 18: 121–133.

Rick, C. M. 1947. Partial suppression of hair development indirectly affecting fruitfulness and the proportion of cross-pollination in a tomato mutant. The American Naturalist 81: 185–202.

Rieseberg, L. H., A. Widmer, A. M. Arntz & J. M. Burke. 2003. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Philosophical Transactions of the Royal Society B 358: 1141–1147. doi 10.1098/rstb.2003.1283.

—— **& J. H. Willis.** 2007. Plant speciation. Science 317: 910–914.

Solbrig, O. T. 1968. Fertility, sterility and the species problem. Pp. 77–96. *In*: V. H. Heywood (ed.), Modern methods in plant taxonomy. Academic Press, London.

Via, S. 2009. Natural selection in action during speciation. Proceedings of the National Academy of Sciences of the United States of America 106: 9939–9946. doi 10.1073/pnas.0901397106.

Waitt, D. E. & D. A. Levin. 1998. Genetic and phenotypic correlations in plants: A botanical test of Cheverud's conjecture. Heredity 80: 310–319. doi 10.1046/j.1365-2540.1998.00298.x.

Waser, N. M. & M. V. Price. 1994. Crossing-distance effects in *Delphinium nelsonii*: Outbreeding and inbreeding depression in progeny fitness. Evolution 48: 842–852.

Whalen, M. D. & G. J. Anderson. 1981. Distribution of gametophytic self-incompatibility and infrageneric classification in *Solanum*. Taxon 30: 761–767.

Williams, D. E. 1985. Tres arvenses Solanáceas comestibles y su proceso de domesticación en el estado de Tlaxcala, México. Master's Thesis. Institucion de Enseñanza e Investigación en Ciencias Agricolas, Chapingo, México.